STUDIES RELATING TO THE METABOLISM OF THE PERIPARTURIENT AND EARLY LACTATING BOVINE

I. Manipulation of Energy Availability for Milk Synthesis in Early to Mid Lactation Holstein Cows Fed Monensin

II. Metabolic Changes of the Periparturient Bovine

by

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Manipulation of Energy Availability for Milk Synthesis in Early to Mid Lactation Holstein Cows Fed Monensin

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INTRODUCTION

Metabolic changes that occur peripartum allow the dairy cow to partition nutrients to the mammary gland in excess of dietary energy intake. Zero net energy balance is not achieved until milk yield is 80 percent of peak yield (about 15 weeks into lactation)(2). During the first month of lactation, the body reserves utilized is equivalent to about 33 percent of the milk produced (2). To minimize the energy deficit, every effort should be made to maximize net energy intake. Various recommendations to improve energy intake include: minimize environmental stress, present a balanced diet to the cow in small amounts at frequent intervals, and increase energy density of the diet. Potentially, another way to reduce energy deficit is to improve the efficiency of rumen fermentation by addition of ionophores.

As reviewed by Schelling (28), and Bergen and Bates(5), ionophores' most accepted modes of action include: modification of ruminal VFA profile by increasing propionate production (27), reducing ruminal methane gas production (32), decreasing ruminal protein deamination thereby increasing protein utilization (16) and increasing apparent digestibilities of energy, NDF, and CP (32).

The influence of monensin on performance of finishing cattle has been summarized by Goodrich et al. (15) using results from about 16,000 cattle. Monensin fed cattle exhibited a 1.6 percent increased average daily gain, 6.4 percent

decreased dry matter (DM) intake, and 7.5 percent decreased feed/gain. Carcass characteristics were not affected.

Data showing the effects of monensin on lactating dairy ruminants are limited. Brown and Hougue (7) reported no change in milk yield, 15 and five percent reduction in milk fat content, and ten and zero percent increase in milk protein content when monensin was fed at 33 and 18 ppm, respectively, to dairy goats during the fourth month of lactation. Feeding monensin four weeks prepartum increased 4% fat-corrected-milk (FCM) immediately postpartum in dairy cows (24). Beede et al. (3) reported no changes in milk yield, percent fat or percent protein in midlactating Holsteins fed lasalocid.

Ionophore supplementation showed no benefits in lactating beef cows (9,14,21), while others (23,26,31) reported increased feed efficiency. Most (9,14,21,26) reported trends toward increased milk yield and/or calf weights with ionophore supplementation. The above trials suggest that ionophores are more likely to be beneficial when energy intake is restricted or when fed to cows during early lactation. The purposes of this study were to determine: 1) a minimum effective dose of monensin, 2) if monensin improves lactational performance in dairy cows in negative energy balance, and 3) if monensin indirectly affects mammary lipogenesis or fatty acid uptake.

MATERIALS AND METHODS

Exp. 1–Dose Titration

Two third lactation Holstein cows with approximately the same bodyweight (BW), stage of lactation, and level of production, were fed a monensin supplemented diet in an increasing and then decreasing step-wise fashion as follows:

EARLY	LATE
Step-up (90 to 118 d postpartum)	Step-down (153 to 174 d postpartum)
Week 1 = 0 monensin	Week 9 = 450 mg/d
Week 2 = 150 mg/d	Week 10 = 300 mg/d
Week 3 = 300 mg/d	Week 11 = 150 mg/d
Week 4 = 450 mg/d	Week 12 = 0 mg/d

Weeks 5-8 = maintained on high (450-600 mg/d) level of monensin. During this time, one cow became lame in the rear feet. No data was used during this 4 wk interval. Both cows were normal when the step-down period started.

Experimental design was a randomized complete block. Data were subjected to split-plot analysis where whole plot was the level of monensin and the sub-plot was stage of lactation. Data were analyzed using the General Linear Models Procedure (20). The statistical model included monensin, cow, (monensin by cow as the whole plot error) and stage of lactation as the subplot effect. If the subplot effect was insignificant ($P>.1$), only main effects are presented. To determine the effects ($P<.1$) of monensin, contrasts (linear, quadratic, and cubic) of drug level

were used.

The monensin supplemented grain mix was fed in an automatic feeder with equal portions fed every two hours. Dry matter intake of the 70:30 corn:soy grain mix was constant at 10.23 kg/d. Alfalfa hay was limited to approximately 2 kg/d. Corn silage was fed ad lib. (approximately 6.6 kg DM/d). Forages were fed twice daily while refusals were collected daily, subsampled, and composited to determine weekly dry matter intake. Approximate forage:concentrate ratio was 45:55.

Nutrient analysis of feedstuffs and average (avg) diet (percent of DM).

Item	Alfalfa Hay	Corn Silage	Grain Mix	Avg Diet
Dry matter	86.08	43.61	87.39	62.0
Crude protein	20.36	9.19	19.04	15.7
ADF	30.39	24.81	4.52	14.4
Calcium	1.32	.48	.70	.7
Phosphorus	.33	.26	.48	.4
Trace mineral salt				.3
Vitamin A (IUx1000/kg)				8.8
Vitamin D (IUx1000/kg)				4.4
Vitamin E (IU/kg)				26.4

Local anaesthesia was used to ruminally fistulate the cows 7 d prior to data collection. Cows were housed in tie stalls and were allowed to exercise 3 h/d.

Milking time was at 0630 and 1830.

Milk, blood, and rumen samples were collected on Tuesday, Thursday, and Saturday of each week. Cows were milked with a bucket milker and samples were collected (a.m. and p.m.) and composited. One sample was used to determine somatic cell count (SCC), fat, protein, lactose and solids-not-fat (SNF) content at the Kansas DHIA processing center. A duplicate sample was heated to 80°C for 2 minutes and stored at -20°C (29) until analysis of fatty acid content. Following a.m. milking, 40 ml of jugular blood was collected in 50 ml heparin coated disposable syringes equipped with 19 gauge needles. Blood was dispensed into NaF treated plastic centrifuge tubes. Ten ml of subcutaneous abdominal venous (SAV) blood was also collected in NaF/$K^2C_2O_4$ treated vacutainers. All blood was mixed and placed on ice for transfer to the laboratory. Blood was centrifuged at 2500 x g (glass tubes) or at 30,000 x g (plastic tubes). Plasma was subsampled and stored in 1.5 ml microcentrifuge tubes. Samples for glucose analysis were stored at -20°C. Plasma (1.4 ml) for insulin and glucagon analysis was treated with 70 ul of benzamidine (11) and stored at -70°C to minimize any proteolytic activity. Jugular and SAV plasma was also stored at -70°C for later analysis of lipoprotein lipase (LPL) activity.

Ruminal contents were mixed, subsampled and strained through 4 layers of cheesecloth after the a.m. milking. Ruminal pH was measured, 25% metaphosphoric acid was added to 5% final concentration (10) before freezing at -

20°C for later VFA analysis.

Bodyweights were measured at 1500 on Tuesday and Wednesday of each week. Weights were averaged for subsequent analysis.

Exp. 2

Eight Holstein cows were supplemented with 200 mg/d of monensin (M) or no monensin (C) for 3 wk. Cows were adjusted to box stalls, diets, and increased handling for 9 d before monensin was added to the diet. Monensin supplementation began 35.6 $\pm$ 2.2 d (mean $\pm$ SE) postpartum. Cows were paired by parity and level of production. The experimental design was a randomized complete block. Data was subjected to split-plot analysis. The whole plot was M vs. C. The sub-plot was wk of trial. Data were analyzed using the General Linear Models Procedure (20). The statistical model included treatment, pair (treatment by pair as the whole plot error) and wk of trial as the sub-plot effect. Weekly means are presented for both treatments, followed by the SE. To determine the effects ($P<.1$) of monensin, contrasts (linear, quadratic, cubic) of wk on trial were used.

Cows were individually fed a 41:59 forage:concentrate (100% DM) total mixed ration 10 percent above ad lib intake twice daily. The total ration was 87.72% DM. A monensin premix (100 mg) was fed after each milking before fresh feed was dispensed.

Composition of the total mixed ration.

Ingredient	% of dry matter
Chopped alfalfa	41.20
Cracked corn	46.97
Soybean meal	7.87
Molasses	2.91
Dicalcium phosphate	.29
Calcium carbonate	.39
Trace mineral salt	.29
Vitamin A, D, and E premix[a]	.08

[a]Added to provide 5433, 3880 and 11 IU/kg of vitamin A, D, and E, respectively.

Nutrient composition of the diet (100% DM basis).

Item	Percent of diet
Crude protein	16.41
ADF	17.12
Calcium	.91
Phosphorus	.46
Potassium	1.59
Magnesium	.13

Feed analysis was done by LSB Products, Manhattan KS 66502.

Cows were milked at 0930 and 2130 while individual stalls were cleaned and bedded. Refused feed was collected daily and subsampled to determine daily DM

intake. The table below illustrates trial design and sampling days.

Trial design and sampling days.

Week 0			Week 1		Week 2		Week 3	
c	c	* * |		* *		* *		* *
d= 3,4		8,9	10	15,16		22,23		27,28

c=Fecal examination--negative for coccidia. Dept. of Laboratory Medicine, Kansas State University College of Veterinary Medicine.

*=Blood, milk, and rumen contents sampled. Bodyweights measured.

|=Start of monensin supplementation.

Milk weights were recorded every milking. Duplicate a.m. and p.m. milk samples were collected and treated the same as exp. 1. Blood collection was the same as previously stated. One additional collection of blood was done 5 min after a 5000 IU sodium heparin challenge into the jugular vein 4 h post-milking. Jugular and SAV blood was collected for analysis of post-heparin releasable lipase activity (30). Blood handling was the same as described above for lipase analysis.

Rumen contents were collected immediately after a.m. milking with a stomach tube. Samples were strained, acidified, and frozen for later analysis. Bodyweights were also measured immediately after a.m. milking.

LABORATORY PROCEDURES

Two steps were used in determination of fatty acid composition in milk--total lipid extraction (12) and saponification and re-esterification. These procedures are detailed by Bitman et al., (6). Heptane was used (instead of hexane) to extract the fatty acid methyl esters. Separation and analysis of fatty acid methyl esters was done with a temperature programmed Hewlett-Packard 5890A gas chromatograph equipped with a 2 mm i.d. x 2 m glass column packed with SP-2330 (Supelco, Inc., Bellefonte, PA) on Chromosorb. Column was conditioned overnight at 200°C. Temperature of the column was held at 120°C for 2 min, then increased at 20°C/min to 160°C, followed by a rate increase of 10°C/min to 185°C (N_2 flow rate: 24.4 ml/min; dual flame ionization detectors, detector temp: 250°C; injection port temp: 235°C). Peak areas were measured with and Hewlett-Packard 3392A Integrator. Peak identities and quantitative accuracy were determined from known standards for each fatty acid (Appendix A).

Frozen rumen content samples were thawed, centrifuged, and analyzed for VFA in a gas chromatograph (17).

Plasma samples were thawed and analyzed for glucose (13), insulin, and glucagon. Radioimmunoassay kits (Cambridge Medical Technology, Inc., Billerica, MA 01861) were used to determine insulin and glucagon concentration in plasma. Glucagon (Lot #258-25J-120) and insulin (Lot #615-70N-80) bovine standards (Lilly Research Laboratories, Indianapolis, IN 46285) were used to validate the assay

against the porcine standards provided in the kits. Option III (the supersensitive protocol) was used on the insulin samples. Glucagon and insulin plasma dilution curves correlated well (104.15% and 103.53%, respectively) with the bovine standard curves. The high correlation coefficients achieved while testing linearity indicates acceptable recovery levels. Sensitivity and 50% binding levels of glucagon and insulin were 68.56, 875.16, and 75.97, 855.64 pg/ml, respectively. Intraassay and interassay CV for glucagon and insulin were 12.16, 18.36, 11.30, and 19.00%, respectively.

Jugular and SAV lipoprotein lipase (LPL) analysis for exp 1 was done at Michigan State University by J. Liesman, under the supervision of R. S. Emery. The procedure was performed in our laboratory for trial #2. A radiolabeled triglyceride (TG) premix was used as a substrate (appendix B). Duplicate plasma (60 ul) or postheparin plasma (20 ul + 40 ul heat denatured serum (HDS) was mixed with 40 ul of 3% BSA (0.15M NaCl pH 7.4), and 100 ul of TG premix. Blank (60 ul HDS), control, fatty acid recovery (100 ul of fatty acid premix or 50 ul of fatty acid premix + 50 ul 8% BSA replaced the 100 ul of TG premix), and quench (100 ul of 8% BSA replaced the TG premix) tubes were also included in each assay. All tubes were incubated at 39°C for 1 h in a shaking water bath. To stop LPL activity, 1.64 ml of heptane:methanol:chloroform (1:1.41:1.25) and 0.425 ml of alkaline buffer (1.266% $K_2B_4O_7 4\ H_2O$, 2.756% K_2CO_3) was added to all tubes on ice. Tubes were then mixed for 15 s, and centrifuged (20°C, 2500 x g, 15 min)

for phase separation to occur. The supranatant (0.5 ml) was dispensed into scintillation vials followed by the addition of 10 ml of scintillation fluid (Bio-Sage II, cat. No. 111195, Research Products International Corp., Mount Prospect, IL 60056-2190). Triglyceride and fatty acid premix (10 ul) was added to the appropriate quench vials immediately prior to the addition of 0.5 ml of quench supranatant and scintillation fluid to make the appropriate standards. The calculations used to determine the FA recovery factor, nmol FA/DPM and nmol FA/tube are in appendix C. The average FA recovery in the supranatant was 26.56%. Precision was acceptable based on the linearity of the graphs in figures 1 and 2. Intraassay and interassay CV were 5.53 and 10.33%, respectively. Specificity was proven by quantitative inhibition of enzyme activity by 1 M NaCl, in combination with serum stimulation and an optimum alkaline pH (22).

RESULTS AND DISCUSSION

Results from exp. 1 and #2 are in tables 1 to 8. The format of table 1 to 4 illustrates the trends that are seen with increasing levels of monensin. If the stage of lactation influenced ($P<.1$) the effect of monensin, both early and late stages are presented. Monensin elicited most of its effects during the initial (150 mg) level. Therefore, monensin levels 150 to 450 mg/d were averaged (table 1). The percent change from level 0 is also presented. Table 5 to 8 includes weekly means, the average value of each trait during the three wk of monensin supplementation, the percent change from the initial week (wk 0=no monensin

supplementation), and the net affect of monensin. Most of the effects of monensin were elicited before wk 2 of trial 2, which partially justifies combining wk 1-3.

Increased milk yield with monensin supplementation has only been shown in one other study (24), when monensin (100 mg) was supplemented to friesian cows 4 wk prepartum to parturition. Other studies previously mentioned showed no milk response to ionophores in mid to late lactating does (7) and cows (3). The consistent response in both trials (6.3 and 7.4%) (tables 1 and 5) suggest that monensin supplementation might be most beneficial during the early lactation energy deficient state. Additional information from exp. 2 is included in figure 3. Weekly means of daily milk production for the eight cows are plotted through the first 12 wk of lactation. The filled arrows indicate the beginning and end of monensin supplementation. The open arrows indicate the same corresponding period for C. Despite blocking by parity and level of production, there was a difference in pretreatment production levels. Within one week after monensin supplementation, M equalled C and surpassed C by 2 wk post-supplementation. Treated cows maintained the edge through the trial. By 3 wk after the trial, M returned back to the same production level relative to C.

The tendency for decreased fat content and yield (-8.6 and -2.8% for exp. 1, and -8.5 and -1.1% for exp. 2, respectively) (tables 1 and 5) agree with others (3,7,24). Armstrong and Blaxter (1) measured a 15% reduction and a 12% increase in milk fat and nitrogen content, respectively, with ruminal propionate infusion at

10% of metabolizable energy requirement in does. Brown and Hougue (7) reported an increase in milk protein content in does only when intake was restricted at neutral energy balance. No change in protein content was seen in midlactating Holsteins fed lasalocid (3). Exp. 1 results show increased milk protein content and yield in early lactation but lower protein content while maintaining daily output in later lactation. Milk protein content did not change during monensin supplementation in exp. 2, but due to higher milk yield, protein yield also increased (tables 1 and 5). The somewhat contradictory results in these trials and past experiments can be explained by the differences in energy status of the cows. In exp. 1, the cows were near the end of the negative energy stage during the step-up period (according to Bauman and Currie (2), zero net energy balance is not achieved until 15 wk or about 105 d postpartum) which ended 118 d after parturition. In exp. 2, all cows were at peak lactation but prior to peak intake. Therefore, the order of energy balance (from lowest to highest) is the cows in: 1)exp. 2, 2)exp. 1-early, and 3)exp. 1-late. During negative energy status, gluconeogenesis from protein is occurring causing a deficiency of amino acids available for milk synthesis. Monensin has been shown to increase nitrogen absorption as a result of lower microbial degradation of feed protein (4). Monensin supplementation also results in increased ruminal propionate which, in theory, causes increased gut usage of propionate, thereby increasing portal supply of glucose (18). Therefore, monensin increases portal supply of glucose and amino acids,

which indirectly increases the supply of substrate needed for milk and protein synthesis. Consequently, the most energy deficient group (exp. 2) responded with no change in milk protein content (+0.2%), but still increased protein output (+10.1%) (table 5); while the cows in exp. 1 during the early stage responded with increased protein content and yield (+8.5 and +14.9%, respectively) (table 1). After positive energy balance is achieved, monensin had no affect on milk protein content (-7.9%) (it is normal for protein content to increase as lactation progresses)(19) and a slight affect on yield (-1.8%) (as a result of maintaining milk yield).

Monensin supplementation did not have a significant affect on fatty acid composition of milk. Based on the control cows in exp. 2, the fatty acid composition of milk shifts to a larger ratio of short chain fatty acids (SCFA) (shorter than C16):long chain fatty acids (LCFA) (longer than C16) (i.e. 0.58 to 0.96) as lactation progresses (table 6). During early lactation, adipose tissue is depleted because of the high energy requirement for milk production beyond the amount supplied by the diet. Adipose tissue fatty acids are mobilized into the bloodstream allowing the mammary gland to incorporate those LCFA of body origin into milk triglycerides. Hence, the high level of LCFA in early lactation milk. After dry matter intake peaks and milk yield begins to decline, adipose tissue is repleted resulting in less preformed fatty acid absorption by the mammary gland. There was no net effect of monensin on SCFA (-2.2%) or LCFA (+7.6%) in exp. 2 (table 6).

The significant increase (+16.7%) in SCFA and decrease (-11.1) in LCFA in milk during the early portion of exp. 1 (table 2) is partially due to advancing lactation as was observed by Chillard et al. (8), who reported decreased LCFA, and increased SCFA and C16 between 1 and 7 wk postpartum. There is a tendency to theorize that monensin may have a positive affect on SCFA content in milk during early lactation. There is insufficient evidence to prove that effect, but the plasma LPL data on the early cows in exp. 1 correlate with the milk fatty acid results. Mammary minus jugular LPL activity decreased 45.2% (table 4) which indicates less LCFA uptake capacity by the mammary gland. However, lack of change of mammary minus jugular LPL (-0.1%) in exp. 2 leaves this theory in doubt (table 8). The large decrease in mammary minus jugular postheparin plasma LPL activity (-70.4%) is unexplainable, especially when no concomitant change occurred in fatty acid composition or plasma lipase activity.

Monensin supplementation did not have a consistent affect on plasma insulin or glucagon (tables 4 and 8). Glucagon tended to decline (0.1 ng/ml) during high doses of M (450 mg/d) in exp. 1, but did not change relative to C on cows in exp. 2. Plasma insulin increased (+14.6%) in exp. 2, but did not change (-3.3%) in exp. 1. Harmon and Avery (18) reported no change in plasma arterial insulin concentration when monensin (220 mg/d) was fed to Holstein steers on an 85% concentrate diet.

Plasma glucose in cows fed M did not change relative to C in exp. 2 (table 8), but tended to increase as lactation progressed (3.19 to 3.40 mM). There was an unexplainable stage by level of monensin interaction in exp. 1. Plasma glucose tended to decline (-0.6%) as dosage increased in the early phase, while increasing in the later stage (+2.2%) (table 4). Due to tight homeostatic mechanisms, insulin:glucagon ratios did not change (+3.9 and +0.9% in exp. 1 and #2, respectively). Harmon and Avery (18) demonstrated an increase in net portal flux of glucose with monensin supplementation. Inconsistency in the results of trials #1 and #2 for glucose, insulin, and glucagon concentrations may be caused from measuring venous plasma concentrations.

Monensin supplementation did not significantly affect dry matter intake in either trial (+13.2 and -1.8%) (tables 3 and 7) which agrees with Bergen (1984) who stated ionophores do not depress intake on high forage diets. Efficiency (4%FCM/DMI) decreased in both M and C groups (-9.0 and -14.7%) in exp. 2 as expected with advancement of lactation, but the net effect of monensin tended to be positive (+5.7%) (table 7).

The fact that monensin increases ruminal propionate concentration is well documented. Both trials support that fact with net increases of 16.4 and 10.7% in exp. 1 and 2, respectively (tables 3 and 7). Isobutyrate is the only other VFA that tended to be affected (+11.1 and +8.1%) by monensin supplementation.

CONCLUSIONS

Evidence from these trials indicate that monensin supplementation at low levels may be economically beneficial to use in lactating cow diets. Many of the results presented are inconclusive due to the low number of experimental units. Additional research is necessary before monensin supplementation can be recommended for commercial use.

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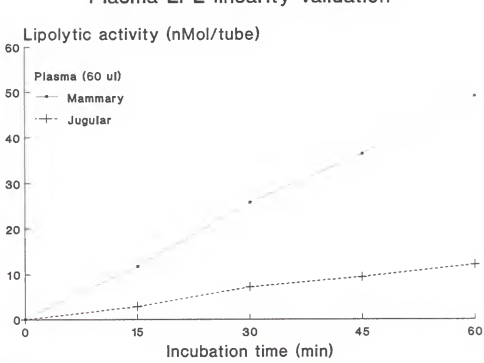


Figure 1

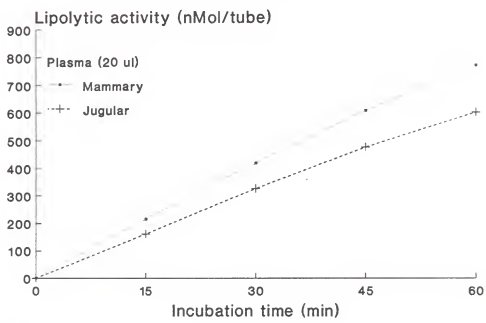
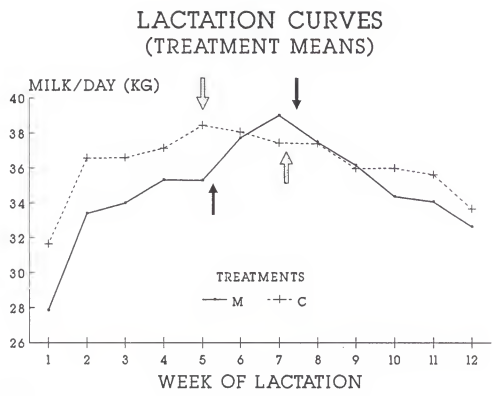


Figure 2


LACTATION CURVES
(TREATMENT MEANS)
MILK/DAY (KG)
40
38
36
34
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28
26
TREATMENTS
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1
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10
11
12
WEEK OF LACTATION

Figure 3

Table 1. Effect of level of monensin on various milk traits.

Trait	Stage	Level of monensin (mg/d) 0	150	300	450	SE	Average level 150-450	Effect of monensin (%)
		Least Square Means						
Milk								
Yield (yld) (kg/d)[2]		18.9	20.0	20.2	20.1	0.3	20.1	+ 6.3
Fat (%)		3.88	3.48	3.59	3.57	0.09	3.55	- 8.6
Fat (kg/d)		0.72	0.69	0.71	0.70	0.03	0.70	- 2.8
4% FCM (kg/d)		18.36	18.36	18.68	18.55	0.61	18.53	+ 0.9
SCM (kg/d)		17.27	17.45	17.77	17.63	0.59	17.62	+ 2.0
Lactose (%)		4.43	4.40	4.44	4.45	0.02	4.43	0.0
Protein (%)[1]	Early	2.80	3.00	3.04	3.07	0.07	3.04	+ 8.5
	Late	3.24	3.04	3.00	2.91	0.07	2.98	- 7.9
Protein (kg/d)[1]	Early	0.56	0.64	0.65	0.64	0.01	0.64	+14.9
	Late	0.57	0.56	0.56	0.56	0.01	0.56	- 1.8
SNF (%)		7.97	7.98	8.02	8.01	0.04	8.00	+ 0.4
SNF (kg/d)		1.50	1.59	1.60	1.60	0.04	1.60	+ 6.4
SCC (x1000/ml)		460	493	382	354	57	410	-10.9

[1]Stage x level of monensin interaction (P<.1).

[2]Monensin effect linear (P<.1).

Table 2. Effect of level of monensin on milk fat characteristics.

Trait	Stage	Level of monensin (mg/d) 0	150	300	450	SE	Average level 150-450	Effect of monensin (%)
		Least Square Means						
Milk fatty acids (% of total fat)								
C8 (%)		2.83	2.92	3.06	3.19	0.20	3.06	+ 8.0
C10 (%)		4.55	4.76	4.96	4.93	0.18	4.88	+ 7.3
C12 (%)[1]	Early	4.41	5.40	5.85	5.72	0.20	5.66	+28.3
	Late	5.23	5.06	4.97	4.89	0.20	4.97	- 4.9
C14 (%)[1]	Early	12.78	13.92	14.98	14.22	0.18	14.37	+12.5
	Late	14.56	14.24	14.04	13.69	0.18	13.99	- 3.9
C16 (%)[1]	Early	33.99	35.50	34.78	32.42	0.33	34.23	+ 0.7
	Late	32.61	31.88	30.89	31.08	0.33	31.28	- 4.1
C16:1 (%)		2.92	2.92	2.90	2.76	0.19	2.86	- 2.1
C18 (%)		12.27	11.22	10.58	10.99	0.58	10.93	-10.9
C18:1 (%)[1]	Early	22.30	20.29	19.57	22.81	0.58	20.89	- 6.3
	Late	22.33	24.40	26.04	25.48	0.58	25.31	+13.3
C18:2 (%)		3.33	2.82	2.95	2.97	0.21	2.91	-12.5
Short chain fatty acids[1]								
C8 + C10 +	Early	24.20	26.94	29.11	28.65	0.79	28.23	+16.7
C12 + C14	Late	27.54	27.05	26.78	26.09	0.79	26.64	- 3.3
Long chain fatty acids[1]								
C18 + C18:1 +	Early	38.84	34.42	33.16	36.03	0.89	34.54	-11.1
C18:2	Late	36.98	38.36	39.50	40.19	0.89	39.35	+ 6.4

[1]Stage x level of monensin interaction ($P<.1$).

[2]Monensin effect linear ($P<.1$).

Table 3. Effect of monensin on bodyweight, feed intake, and ruminal parameters.

Trait	Stage	Level of monensin (mg/d) 0	150	300	450	SE	Average level 150-450	Effect of monensin (%)
		----------Least Square Means----------						
Bodyweight (kg)		509	530	568	565	32	554	+ 8.9
Dry matter intake (kg/d)		15.1	15.7	18.1	17.5	1.7	17.1	+13.2
Ruminal								
pH		5.60	5.99	6.32	6.27	0.35	6.19	+10.6
Acetate (mM)		68.8	69.9	70.9	69.8	2.0	70.2	+ 2.0
Propionate (mM)[2]		21.1	23.6	23.6	26.5	0.8	24.6	+16.4
Acetate:Propionate[2]		3.33	3.00	3.05	2.68	0.5	2.91	-12.6
Butyrate (mM)		14.1	13.8	15.3	13.8	0.8	14.3	+ 1.4
Isobutyrate (mM)		1.29	1.30	1.53	1.47	0.07	1.43	+11.1
Valerate (mM)		1.39	1.42	1.49	1.45	0.07	1.45	+ 4.6
Isovalerate (mM)		2.36	2.34	2.68	2.46	0.20	2.49	+ 5.7
Total VFA (mM)		108.7	113.0	115.2	115.8	3.6	114.7	+ 5.5

[1]Stage x level of monensin interaction (P<.1).

[2]Monensin effect linear (P<.1).

Table 4. Effect of monensin on various plasma parameters.

Trait	Stage	Level of monensin (mg/d) 0	150	300	450	SE	Average level 150-450	Effect of monensin (%)
		----------Least Square Means----------						
Plasma								
Insulin (ng/ml)		0.82	0.81	0.76	0.81	0.05	0.79	- 3.3
Glucagon (ng/ml)[2]		0.97	0.95	0.97	0.86	0.03	0.93	- 4.5
Insulin:Glucagon ratio		0.85	0.88	0.79	0.98	0.06	0.88	+ 3.9
Glucose (mM)[1]	Early	3.56	3.60	3.60	3.42	0.04	3.54	- 0.6
	Late	3.39	3.41	3.41	3.57	0.04	3.46	+ 2.2
Plasma LPL (nMol FA released/h/ml)								
Mammary	Early	1271	874	703	653	169	743	-41.5
	Late	764	837	766	794	169	799	+ 4.6
Jugular	Early	231	191	161	170	24	174	-24.7
	Late	170	201	180	155	24	179	+ 5.1
Mammary-Jugular	Early	1040	684	542	483	147	570	-45.2
	Late	595	636	586	639	147	620	+ 4.3

[1]Stage x level of monensin interaction (P<.1).

[2]Monensin effect linear (P<.1).

Table 5. Effect of monensin on various milk traits.

Trait	Treat-ment	Week of trial 0	1	2	3	SE	Average from Wk 1-3	Change wk 0 (%)	Net effect of monensin (%)
		--Least Square Means--							
Milk									
Yield (yld)*	M[2]	34.9	36.9	39.7	39.4	1.0	38.7	+10.9	+ 7.4
(kg/d)	C	37.0	37.5	38.7	38.7	1.0	38.3	+ 3.5	
4% FCM	M	32.5	33.6	32.2	33.7	1.2	33.2	+ 2.2	+ 3.6
(kg/d)	C	34.7	35.5	33.5	33.5	1.2	34.2	- 1.4	
Fat	M	3.48	3.13	2.84	2.96	0.09	2.98	-14.4	- 8.5
(%)	C	3.51	3.56	3.25	3.10	0.09	3.30	- 5.9	
Fat yld[2]	M	1.23	1.21	1.11	1.18	0.05	1.17	- 4.9	- 1.1
(kg/d)	C	1.31	1.35	1.22	1.20	0.05	1.26	- 3.8	
Protein	M	3.00	2.89	2.83	2.88	0.04	2.87	- 4.3	+ 0.2
(%)	C	3.08	2.92	2.92	2.99	0.04	2.94	- 4.5	
Protein yld	M	1.05	1.11	1.10	1.15	0.04	1.12	+ 6.7	+10.1
(kg/d)	C	1.16	1.11	1.10	1.15	0.04	1.12	- 3.4	
SCC	M	125	136	68	84	28	96	-23.2	- 5.1
(x 1000/ml)	C	94	84	61	86	28	77	-18.1	

*Mean is calculated from all days in week. All other traits were measured during the final two days of each week.

[1]Treatment x wk interaction (P<.1).

[2]Weekly effect is linear (P<.1).

[3]Weekly effect is quadratic (P<.1).

Table 6. Effect of monensin on milk fat characteristics.

Trait	Treat-ment	Week of trial 0	1	2	3	SE	Average from Wk 1-3	Change wk 0 (%)	Net effect of monensin (%)
		--Least Square Means--							
Milk fatty acids (% of total fat)									
C8[2]	M	3.42	3.30	3.42	4.60	0.16	3.77	+10.2	+ 1.2
(%)	C	3.67	3.43	3.79	4.79	0.16	4.00	+ 9.0	
C10[2]	M	3.82	4.14	4.19	4.82	0.16	4.38	+14.7	+ 0.8
(%)	C	4.11	4.11	4.65	5.28	0.16	4.68	+13.9	
C12[2]	M	3.95	4.39	5.03	5.46	0.18	4.96	+25.6	- 5.1
(%)	C	4.14	4.33	5.62	6.27	0.18	5.41	+30.7	
C14[2]	M	11.75	12.44	13.32	13.07	0.31	12.94	+10.1	- 3.4
(%)	C	11.65	12.45	13.38	13.82	0.31	13.22	+13.5	
C16[2]	M	32.59	34.40	35.12	34.71	0.81	34.74	+ 6.6	- 7.2
(%)	C	31.58	34.25	37.24	36.37	0.81	35.95	+13.8	
C16:1[2]	M	4.89	3.83	3.14	2.25	0.26	3.07	-37.2	- 6.9
(%)	C	3.99	3.49	2.73	2.11	0.26	2.78	-30.3	
C18[2]	M	11.26	9.03	8.27	8.95	0.69	8.75	-22.3	- 5.8
(%)	C	11.79	10.95	9.61	8.99	0.69	9.85	-16.5	
C18:1[1]	M	24.93	24.27	23.73	22.39	0.63	23.46	- 5.9	+13.1
(%)	C[2]	24.73	22.71	19.00	18.36	0.63	20.02	-19.0	
C18:2	M	2.71	2.83	3.05	3.01	0.10	2.96	+ 9.2	+ 9.8
(%)	C	3.25	3.38	3.14	3.16	0.10	3.23	- 0.6	
C18:3	M	0.67	0.85	0.73	0.77	0.06	0.78	+16.4	+28.3
(%)	C	1.01	0.89	0.94	0.85	0.06	0.89	-11.9	
Short chain fatty acids[2]									
C8+C10+	M	22.94	24.27	25.96	27.95	0.49	26.06	+13.6	- 2.2
C12 + C14	C	23.58	24.33	27.43	30.16	0.49	27.31	+15.8	
Long chain fatty acids[2]									
C18+C18:1	M	39.57	36.97	35.78	35.12	1.20	35.96	- 9.1	+ 7.6
C18:2+C18:3	C	40.78	37.92	32.69	31.36	1.20	33.99	-16.7	

[1]Treatment x wk interaction (P<.1).

[2]Weekly effect is linear (P<.1).

[3]Weekly effect is quadratic (P<.1).

Table 7. Effect of monensin on bodyweight, feed intake and ruminal parameters.

Trait	Treat-ment	Week of trial 0	1	2	3	SE	Average from Wk 1-3	Change wk 0 (%)	Net effect of monensin (%)
		--Least Square Means--							
Dry matter*[2]	M	19.8	21.6	22.6	23.1	0.8	22.4	+13.1	- 1.8
intake (kg/d)	C	21.3	23.0	25.1	25.3	0.8	24.5	+14.9	
Bodyweight[2]	M	580	586	598	603	5	596	+ 2.8	+ 0.9
(kg)	C	565	562	580	586	5	576	+ 1.9	
Adjusted[2]	M	3.52	3.86	3.81	3.93	0.11	3.87	+ 9.9	+ 1.4
intake(%BW)	C	3.98	4.30	4.28	4.37	0.11	4.32	+ 8.5	
Efficiency[2]	M	1.55	1.40	1.41	1.43	0.06	1.41	- 9.0	+ 5.7
(4%FCM/DMI)	C	1.56	1.38	1.33	1.28	0.06	1.33	-14.7	
Ruminal									
Acetate[1]	M[3]	55.5	47.7	50.7	62.2	4.1	53.5	- 3.6	- 3.8
(mM)	C	55.7	58.7	57.0	51.8	4.1	55.8	+ 0.2	
Propionate[2]	M	19.2	20.7	24.7	27.8	1.8	24.4	+27.1	+10.7
(mM)	C	18.3	18.8	23.3	21.8	1.8	21.3	+16.4	
Butyrate	M	9.69	9.05	8.66	11.43	0.94	9.71	+ 0.2	- 1.5
(mM)	C	9.18	10.02	8.60	9.40	0.94	9.34	+ 1.7	
Isobutyrate	M	1.15	1.02	1.03	1.25	0.11	1.10	- 4.3	+ 8.1
(mM)	C	1.29	1.29	1.08	1.01	0.11	1.13	-12.4	
Valerate	M	1.24	1.20	1.36	1.60	0.12	1.39	+12.1	+ 2.3
(mM)	C	1.12	1.17	1.28	1.23	0.12	1.23	+ 9.8	
Isovalerate	M	1.60	1.54	1.42	1.83	0.19	1.60	0.0	-11.3
(mM)	C	1.68	1.74	1.38	1.34	0.19	1.49	-11.3	
Ac:Pro	M	3.07	2.52	2.23	2.39	0.65	2.38	-22.5	-10.3
ratio	C	3.12	3.18	2.50	2.55	0.65	2.74	-12.2	
Total VFA[1]	M[2]	88.42	81.18	87.86	106.12	6.07	91.72	+ 3.7	+ 0.3
(mM)	C	87.30	91.69	92.62	86.49	6.07	90.27	+ 3.4	

*Mean is calculated from all days in week. All other traits were measured during the final two days of each week.

[1]Treatment x wk interaction ($P<.1$).

[2]Weekly effect is linear ($P<.1$).

[3]Weekly effect is quadratic ($P<.1$).

Table 8. Effect of monensin on various plasma parameters.

Trait	Treat-ment	Week of trial 0	1	2	3	SE	Average from Wk 1-3	Change from wk 0 (%)	Net effect of monensin (%)
		--Least Square Means--							
Plasma									
Glucose[2]	M	3.19	3.33	3.41	3.30	0.07	3.35	+ 5.0	- 2.8
(mM)	C	3.19	3.34	3.50	3.49	0.07	3.44	+ 7.8	
Insulin[2]	M	0.55	0.62	0.64	0.65	0.03	0.64	+16.4	+14.6
(ng/ml)	C	0.57	0.57	0.59	0.58	0.03	0.58	+ 1.8	
Glucagon	M	1.32	1.37	1.45	1.44	0.09	1.42	+ 7.5	+ 2.1
(ng/ml)	C	1.68	1.67	1.89	1.76	0.09	1.77	+ 5.4	
Ins:Glucagon	M	0.49	0.49	0.47	0.48	0.03	0.48	- 2.0	+ 0.9
ratio	C	0.34	0.34	0.31	0.33	0.03	0.33	- 2.9	
Plasma LPL (nMol FA released/h/ml)									
Mammary[2]	M	964.5	770.2	765.7	801.8	88.6	779.2	-19.2	- 0.9
	C	1167.3	961.0	981.4	919.1	88.6	953.8	-18.3	
Jugular	M	281.2	243.0	264.4	252.6	31.6	253.3	- 9.9	- 2.9
	C	336.6	295.8	319.9	323.2	31.6	313.0	- 7.0	
Mammary -	M	683.3	527.2	501.3	549.2	64.7	525.9	-23.0	- 0.1
Jugular	C	830.6	665.1	661.5	595.9	64.7	640.8	-22.9	
Postheparin plasma LPL (uMol FA released/h/ml)									
Mammary	M	42.33	26.99	33.03	35.21	3.79	31.74	-25.0	-21.8
	C	41.34	36.89	41.51	40.67	3.79	39.69	- 4.0	
Jugular	M	34.03	23.38	32.75	33.04	3.62	29.72	-12.7	- 8.9
	C	35.87	31.14	37.13	35.22	3.62	34.50	- 3.8	
Mammary -	M	8.30	3.61	0.28	2.17	2.51	2.02	-75.7	-70.4
Jugular	C	5.48	5.75	4.38	5.45	2.51	5.19	- 5.3	

[1]Treatment x wk interaction ($P<.1$).

[2]Weekly effect is linear ($P<.1$).

[3]Weekly effect is quadratic ($P<.1$).

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Metabolic Changes of the Periparturient Bovine

J. C. Kube, J. E. Shirley, D. L. Harmon, and R. A. Frey

INTRODUCTION

Parturition and the initiation of lactation are two physiological states that require enormous redirection of nutrient flow in order to meet the added metabolic requirements (2). To better define the requirements during these conditions, researchers must understand the controlling mechanisms of parturition and lactogenesis.

Normal hormonal (and enzymatic) changes associated with parturition and initiation of lactation have been reviewed (1,6,8,11,15,29,33,34,36). By understanding endocrine changes that occur peripartum, lactation has been successfully reinitiated in hypophysectomized does (9) and induced in 7.5 mo pregnant heifers (35). Induction of parturition is easily performed in various species with high predictability and few complications, but milk yield has been less than normal.

Despite modest success with induction of lactation and parturition, understanding the homeorhetic mechanisms that are responsible for the shift of nutrient flow from body maintenance, to the fetus, and finally to the mammary gland are necessary for further advancements to be made. One organ that appears to dictate the direction of nutrient flow is the mammary gland. To understand the extent of control the mammary gland has on body metabolism, one must determine whether the nutrient redistribution associated with lactation is due to enhanced

mammary gland activity and milk removal, to hormonal changes associated with parturition, or both. The objective of this experiment was to determine if selected metabolic and hormonal changes normally associated with parturition and initiation of lactation result from parturition, lactation, or both.

MATERIALS AND METHODS

Eleven Hereford x Angus cows were bilaterally mastectomized (M) during the sixth to seventh month of their second gestation at the Department of Surgery and Medicine, Kansas State University College of Veterinary Medicine. Fourteen normal Hereford x Simmental multiparous cows were controls (C). Blum et al. (4) stated that the effect of breed (Simmental, Brown Swiss, Holstein, or Holstein x Simmental) on blood parameters is rarely significant. All cows were group fed 4.5 kg (starting 1 mo prepartum until parturition) or 6.8 kg (from parturition until 2 mo postpartum) cracked corn-soy concentrate plus ad lib sorghum silage. Jugular and subcutaneous abdominal venous (SAV) blood samples were collected between 0900 and 1100 on -30, -5, -4, -3, -2, and -1 d from expected calving date and 0, 1, 2, 3, 4, 5, 10, 15, 21, 25, 30, and 55 d from actual calving date. Subcutaneous adipose tissue samples were collected -21 and -5 d from expected calving date and 5, and 21 d from calving. An additional adipose tissue sample was collected 55 d postpartum on M. Calves were weaned from C on 28 d postpartum.

Experimental design was a completely randomized design with repeated measures over time. Data were analyzed using the General Linear Models

Procedure (19). Whole plot (M vs. C) effects were determined with cow within treatment as the error term. Whole plot SE are included in the results. To determine if treatments are different ($P<.05$) at a particular point, multiply the SE by 4. If the difference between the two points is greater that sum, then the difference is significant ($P<.05$).

All cows were fully recovered from the mastectomy surgery prior to sample collection. Jugular and SAV blood was collected, transported to the laboratory in an ice bath, and processed for later analysis of lipoprotein lipase (LPL), glucose, insulin (INS), and glucagon (GLUCAG) identical to the procedure described in part I of this thesis. Plasma samples that were stored for analysis of cortisol (CORT), thyronine (T3), thyroxin (T4), and prolactin (PRL) were processed the same as the samples to be analyzed for glucose. Subcutaneous adipose tissue was taken by surgical biopsy 20 cm lateral to midline in the loin area(29). The biopsy site was clipped, scrubbed, and anesthetized with 7 ml of Lidocaine-HCl. A 4 cm incision, parallel to the spine, was made and approximately 1 gm of adipose tissue was excised. The tissue was immediately placed in 0.15 M KCl (5°C) and transported to the laboratory on ice. The incision was sutured with no. 1 Vetafil. Subsequent samples were collected from the opposite loin area as well as both sides of the dorsal shoulder region 20 cm lateral to the midline. In the laboratory, the adipose tissue was rinsed with 0.15 M KCl through 4 layers of cheesecloth and blotted dry to remove blood. Samples were placed in labeled twirl packs and stored at -70°C

until further analysis.

Laboratory procedures for plasma analysis of glucose, INS, and GLUCAG are described in part I. These samples were pooled with the samples from part I of thesis for analysis, therefore, performance data is the same. Radioimmunoassay kits (Cambridge Medical Technology, Inc., Billerica, MA 01861) were also used to determine plasma CORT, T4, and T3 concentrations. The kits were previously evaluated against bovine standards as explained in the protocol included with the kits. Sensitivity levels of CORT, T4, and T3 were 1.4 ng/ml, 0.5 ng/ml, and 54.7 pg/ml, respectively. Intraassay and interassay CV for CORT, T4, and T3 were 14.92 and 15.35%, 6.75 and 12.60%, and 7.51 and 8.78%, respectively. Evaluation of plasma PRL was performed at Michigan State University under the supervision of H. A. Tucker. Sensitivity level of PRL was 0.3 ng/ml at 90% binding. All samples were evaluated in a single assay, with intraassay CV = 8.35%.

Jugular and SAV LPL analysis has been described in part I. Adipose tissue LPL protocol is identical to the plasma protocol. See appendix D for the description of tissue preparation for LPL analysis (29). The filtrate was used for protein determination and lipolytic activity. Adipose tissue LPL activity is expressed as the amount of fatty acids released/(h mg) protein. Intraassay CV (all samples were run in one assay) for adipose tissue LPL was 10.14%. Precision was acceptable and lipolytic activity remained constant through 90 min (figure 1). An incubation time of 60 min with a filtrate vol of 60 ul was identical to the time and

vol used on the plasma. Average FA recovery was 29.23%. Protein concentration of the filtrate was determined with BCA Protein Assay kit (Pierce, Rockford, IL 61105) (31). The standard protocol was followed using BSA to make 6 standards ranging from 200 to 1200 ug/ml of protein. Diluent used was 0.15M KCl. Intraassay and interassay CV of protein content was 4.39 and 5.80%, respectively.

RESULTS AND DISCUSSION

All parameters measured are illustrated in graphic form (figures 2-19). Within each measurement, there are two graphs. The top graph shows weekly changes from 7 wk before through 8 wk after parturition. Data on the control cows were collected through -3 through 5 wk only. The bottom graph shows daily changes from 15 d before through 15 d after parturition. Mastectomized cows were artificially inseminated, while C were naturally serviced. Therefore, calving dates on M were more accurate, allowing more precise data collection prior to calving (no samples taken -15 to -10 d prepartum). Standard error bars represent whole plot SE.

Glucose

Glucose levels were higher in the M the week of, through 3 wk after parturition (figure 2). Weaning the calves from C tended to increase plasma glucose levels to that of M. The daily trend of glucose concentration around parturition is similar between groups (figure 3). However, plasma glucose is higher in M on -1, 1, 2, 3, 4, and 5 d form calving. Davis et al. (11) (doe) and Vaios and

Phillips (36) (cow) both report similar increases in plasma glucose 1 d prepartum and return back to normal 1 d postpartum. The reason for the elevated glucose levels in M may be from lack of glucose uptake by the mammary gland. Davis et al. (11) measured increased mammary glucose uptake starting 2 d prepartum, continuing through parturition and lactation. The triggering mechanism for increased glucose uptake is the precipitous drop in plasma progesterone concentration 2 d prepartum. Progesterone blocks lactose synthetase function to convert glucose + UDP-galactose --- lactose (Stevenson personal communication, 1988). Therefore the overall elevation of plasma glucose in M is a result of gland removal, however, the glucose spike at parturition is independent of lactogenesis and may be a result of epinephrine release at parturition.

Insulin

Plasma INS levels tend to be elevated in M through parturition until 2 wk postpartum (figure 4) then returns to prepartum levels by 2 wk postpartum. Beginning 1 wk prepartum, INS concentration increased and remained high throughout data collection in C. In contrast, deBoer et al. (12) reported a decrease in plasma INS between measurements at 2 wk prepartum and 3 wk postpartum in Holsteins. Blum et al. (5) also found an insignificant numerical decrease in plasma INS from 3-4 wk prepartum to 3-4 wk postpartum in Jerseys. Insulin concentration spiked upward in both groups on the day before parturition (figure 5). A similar surge was reported by Chew et al. (6) in dairy cows, but the surge began the day

of calving. Plasma INS remained significantly higher in M through 4 d after calving. Malven et al. (21) recorded an increase in arterial INS from -7 to -4 d, then decreased at a constant rate until 4 d postpartum. Concurrently, blood flow to the mammary gland was lowest on -4 d, and highest on 4 d from parturition. Malven et al. (21) concluded that net mammary uptake of INS changed very little from -7 d to 6 d from calving.

The somewhat contradictory results between this and past trials could be due to the animals studied. Beef cows have not been intensely selected for milk production like dairy animals. Also, the calves from C did not remove all the milk from their dams during the first days after birth. This incomplete milk out may have influenced blood flow and INS (and glucose) uptake by the mammary gland, which could result in higher plasma INS and glucose concentrations. Another possibility for increased levels of plasma glucose and INS may be from the increased energy level of the diet fed after calving.

Glucagon

Plasma GLUCAG was elevated in M from -1 to 3 wk from parturition (figure 6). Controls tended to have lowest GLUCAG levels -4 to 4 d from calving, but surged at -3 d and returned to the prepartum low level by 1 d from calving (figure 7). A subtle surge was also detected in M at -4 d, continued increasing until 4 d from calving, then leveled off. Increased GLUCAG is a gluconeogenic signal to the liver to increase plasma glucose levels (13), thereby causing increased

INS levels as was seen in previous figures.

Insulin:glucagon ratio

Plasma INS:GLUCAG ratio was higher in M -3 wk and continued to be numerically higher through 2 wk from parturition (figure 8). The general pattern of changes was similar between groups. Daily INS:GLUCAG levels mimicked one another very closely (figure 9). Both groups displayed a downward surge beginning -3 d and reached a nadir -2 and -1 d from calving in M and C, respectively. Controls peaked on 0 d and dropped precipitously until 3 d from calving, while M tended to remain at a relatively lower peak than C from 0 to 3 days from calving--after which levels dropped until 5 d postpartum. Because of strict homeostatic control, INS:GLUCAG ratio illustrated very similar patterns. Parturition itself is responsible for the major changes of these hormones, with lactation having a secondary effect. Lack of lactation in M tended to cause a lag in recovery of hormones back to prepartum levels mainly due to the absence of a milk synthesis drag.

Cortisol

Plasma CORT levels were higher throughout the periparturient period in M. Periphery levels of CORT were significantly higher in M at -3 wk and declined at a faster rate than C until 1 wk from parturition (figure 10). Both groups tended to increase in CORT until 3 wk, then merged together by 5 wk from calving (possibly due to weaning the calves from C). Daily CORT levels were also higher

in M. Inconsistent surges began -4 d and continued 3 d from parturition in both groups (figure 11).

Glucocorticoid (GCC) is considered to be one of the lactogenic hormones, except in rabbits. Its functions include playing roles in differentiation of rough endoplasmic reticulum and golgi apparatus (23). In late gestation, mammary cell receptors for GCC increase, and competitive binding of progesterone on GCC receptors is removed (Stevenson personal communication, 1988). Cortisol binding globulin also decreases peripartum, allowing increased metabolically active free GCC (34). Increased binding of plasma GCC to mammary gland receptors may explain the decrease in plasma cortisol concentration immediate prepartum and shortly postpartum. The lack of mammary receptors in M may explain the elevated CORT levels throughout the collection period. The surge of CORT around parturition is similar to Smith et al. (30), only the spike of CORT at parturition was more distinct in their study. They (30) conclude that it is not known whether the surge at parturition is necessary for lactogenesis or whether it is stress related. These results indicate that it may be stress related because the surge occurs in M and C, irregardless of lactogenesis.

Thyronine and thyroxin

Plasma T4 was higher -3 wk, while T3 and T4 were higher -1 wk from parturition (figures 12 and 14). Thyronine and T4 increased in both groups the wk before calving, and declined the wk after calving except T3 remained elevated in

M. After 3 wk postpartum, both groups were similar in plasma thyroid hormone concentration. Daily changes in thyroid hormone concentrations were very similar. Mastectomized cows tended to lag behind C when the hormones spiked around parturition (figures 13 and 15). Plasma T3 spike occurred -3 d in C, but not until 2 to 3 d from parturition in M. The lag in plasma T4 was only 1 d later for M (C peaked 0 d, and M peaked 1 d from parturition) than C. The T3 spike was more pronounced and of greater magnitude in C relative to M, whereas, the opposite was true for T4.

Blum et al. (3) reported that plasma T3 is highly correlated (r=.72) to energy and nitrogen balance in sheep. Elevated T3 may contribute to increased blood flow to the mammary gland (10) as well as increased metabolic activity (T4 is considered a prohormone of T3)(18). The lag in the thyroid hormone spike in M may be a result of the lack of need for nutrients and energy needed for milk synthesis. The fact that the spike still occurs in M indicates that the spike is induced by parturition. An additional function of thyroid hormones is its synergistic effect it has with PRL and its ability to stimulate synthesis of a-lactalbumin (a protein subunit of the lactose synthetase complex)(Stevenson personal communication, 1988).

Prolactin

The weekly trend for plasma PRL is similar in both M and C (figure 16). Plasma PRL declines from -3 wk to a low at 1 wk from calving for M, while C doesn't reach its low until 5 wk postpartum. The low at 1 wk postpartum is

attributed to a lack of milk synthesis in M, while the low in C at 5 wk postpartum is attributed to weaning the calves, which also stopped milk synthesis. Daily PRL concentrations were elevated in both groups at -5 d then dropped to a low at -3 and -2 d from calving for M and C, respectively (figure 17). Both groups surged to a peak at -1 d then declined rapidly to below prepartum levels by 1 and 2 d from parturition, respectively. Controls had higher levels from 5 to 15 d postpartum. Similarly, Davis et al. (11) showed PRL started to increase -2 d, peaked and returned to normal levels by 1 to 2 d from calving. Erb (15) reports that PRL concentration was similar 3 d before and 1 to 3 d after calving. The PRL surge is approximately the same magnitude of duration as the length of calving, therefore PRL may be a stress hormone (Stevenson personal communication, 1988).

Prolactin is influenced by day length, with higher peripheral levels in the summer (18). The unusually high values (up to 3 times) recorded in the trial are attributed to the stress of sampling. Samples were taken in a head catch due to the nature of the cows used. Prolactin is not required after parturition to maintain lactation, but has been shown by Cowie (9) to enhance lactation. Goats that were hypophysectomized in mid lactation dropped drastically in milk yield to minimal levels. Hormone replacement therapy (GCC + T3 + growth hormone) resulted in 28% of normal milk yield. Addition of PRL to the regimen resulted in 100% recovery in milk yield. There is an increased number of PRL receptors in the mammary gland after parturition (34). Progesterone has been shown to block PRL

ability to induce synthesis of PRL receptors (14). This may explain the lower plasma PRL levels during calving. Akers et al. (1) reported that low PRL levels impairs cellular function but does not reduce epithelial cell numbers. CB154 (an ergot alkaloid) was used to block PRL secretion (1). Prolactin is suspected of having a positive influence on intestinal weight (20). Other functions of PRL include: regulation of casein mRNA synthesis, by increasing transcription rates and increasing half-life of casein mRNA in mammary tissue explants from mid pregnant rats (17,22,27). Goodman et al. (16) concluded that PRL induces synthesis and release of a-lactalbumin (protein subunit of the rate limiting enzyme for lactose synthesis). Estrogen and GCC potentiate the lactogenic effect of PRL if progesterone is absent.

Mammary Minus Jugular Lipoprotein Lipase

Plasma M-JLPL began to increase -1 wk and peaked at 1 wk from parturition in C (figure 18). Lipase levels remained high through 3 wk after calving, then dropped by 5 wk in C, possibly due to weaning the calves at 4 wk after birth. Similar increases (10x) when MLPL activity were measured at -3 and 2 week from date of caesarean (essentially start of lactation) (28). Daily levels of plasma M-JLPL began to rise -1 d, peaked 1 d, and declined slightly 2 d after calving in C (possibly a result of incomplete milk removal of the newborn calves) (figure 19). Enzyme activity remained elevated 3 through 15 d after calving. The lack of lipase activity in M indicates that mammary LPL is independent of direct hormonal control

in the bovine. The minimal level of lipolytic activity in M indicates complete removal of the mammary glands was achieved and further substantiates the use of plasma M-JLPL activity as an indication of mammary tissue activity.

Adipose Tissue Lipoprotein Lipase

Milk LPL activity has been reported to be 12-16 times that of adipose tissue (26). Zinder et al. (37) indicated that LPL activity increased in the mammary gland and decreased in adipose tissue when PRL was infused intravenously. Even though enzyme activity levels are generally much higher in the mammary gland than in adipose tissue, the mammary gland lacks the response mechanism present in adipose tissue (26). Three liters of glucose (25% w/v) were infused over a 24 h period to lactating Holstein cows. Subcutaneous adipose LPL activity increased, while milk LPL showed an insignificant numerical decrease (25). Figure 20 illustrates that adipose tissue lipoprotein lipase (ALPL) activity was higher in M than C at -3 wk from calving. Enzyme activity in M dropped 75% by -1 wk, and stayed low until 1 wk from parturition, then gradually increased through 6 wk. The reduction in ALPL around parturition in M is a result of hormonal changes associated with parturition and is independent of mammary gland function. Adipose tissue LPL activity was minimal beginning -3 wk through 1 wk from parturition, then increased to M levels by 3 wk. The increase may have been a result of the higher energy diet fed to all cows after calving. Controls were larger cows and may not have been receiving sufficient dietary energy prepartum to accumulate adipose tissue like the

smaller M cows. Tanaka et al. (32) reported increased ALPL in goats when high concentrate diets were fed. The higher ALPL levels correlates well with the higher levels of glucose, INS, and GLUCAG measured in M compared to C. Shirley et al. (28) found a 33% reduction in ALPL with the onset of lactation. Similar results were recorded with goats when omental fat was sampled -7 to 3 wk from parturition (7).

CONCLUSIONS

The absence of lactogenesis affected all parameters measured to a degree. The major effects were recorded in the metabolic hormones (INS and GLUCAG), in that failure of the milk drain prolonged the parturition peaks. Homeostasis is tightly controlled and concentration soon returned to prepartum levels. Other effects that removal of the mammary gland may have had were a decrease in number of receptors for GCC binding causing higher GCC levels to be recorded throughout the collection period. Also, mastectomy was responsible for delayed T3 and T4 peaks. Alternately, gland removal had little effect on PRL concentration. The high levels (up to 3 times normal) may have influenced these results. Finally, lipase activity responded in a highly predicable fashion.

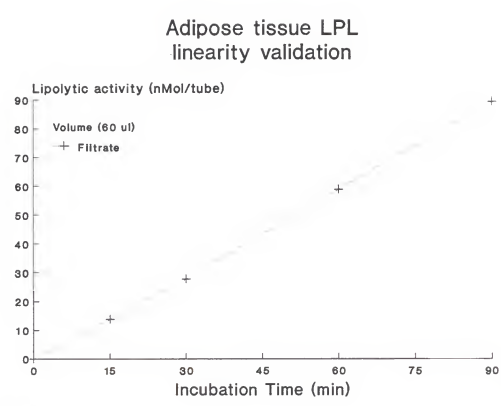
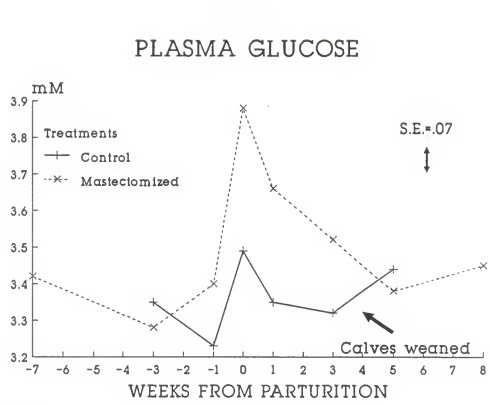

Adipose tissue LPL
linearity validation
Lipolytic activity (nMol/tube)
Volume (60 ul)
Filtrate
90
80
70
60
50
40
30
20
10
0
0
15
30
45
60
75
90
Incubation Time (min)

Figure 1



TRT*WK (P(.01)
Figure 2.

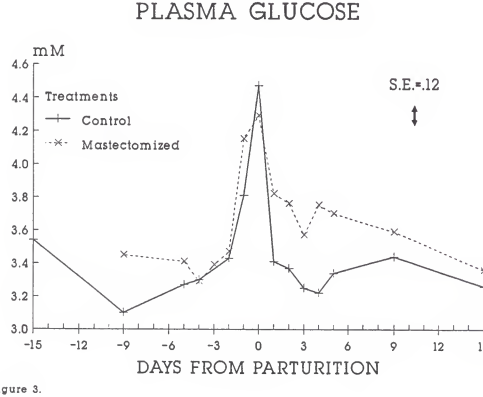


Figure 3.

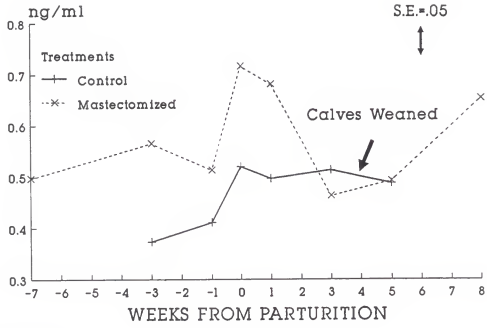


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Figure_4.

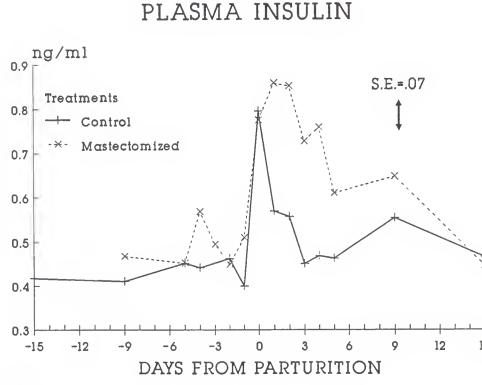
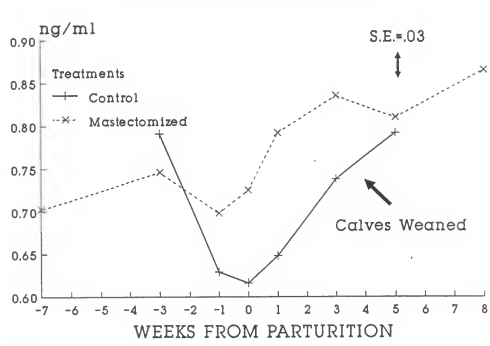


Figure 5.



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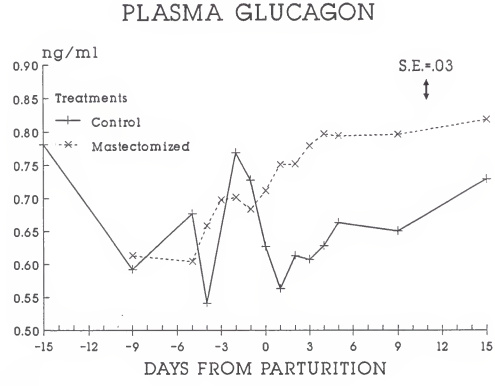

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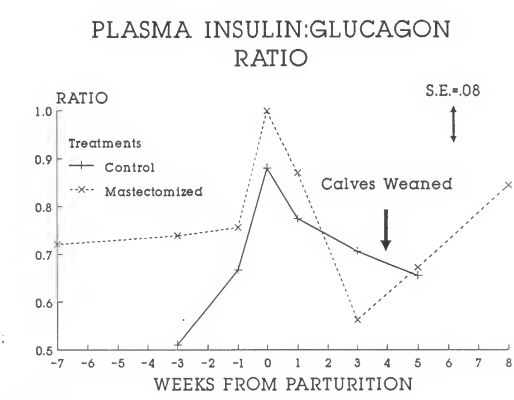

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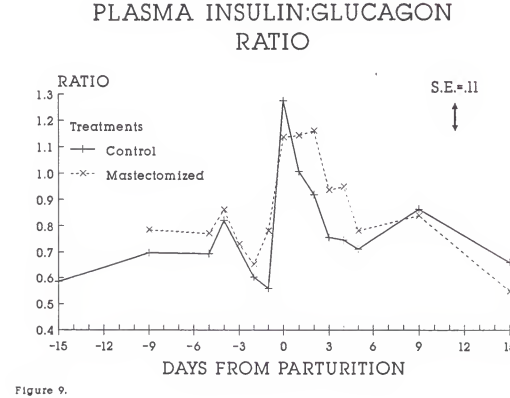

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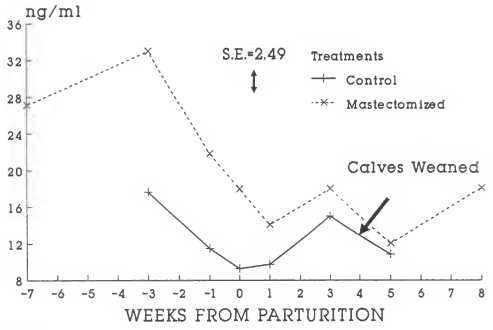
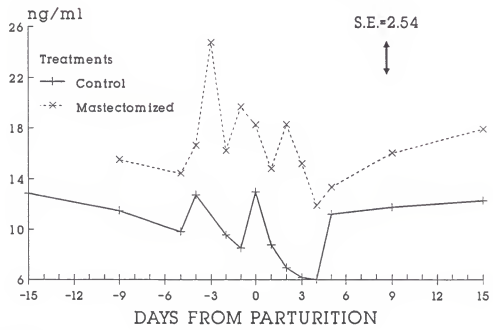
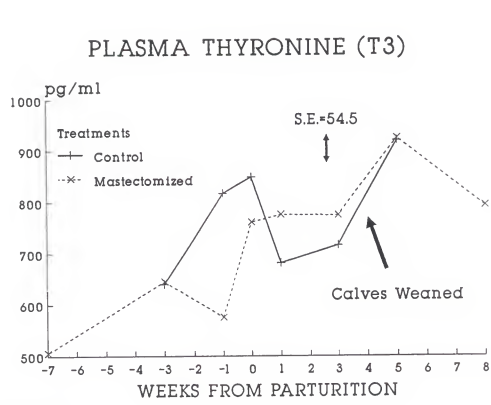


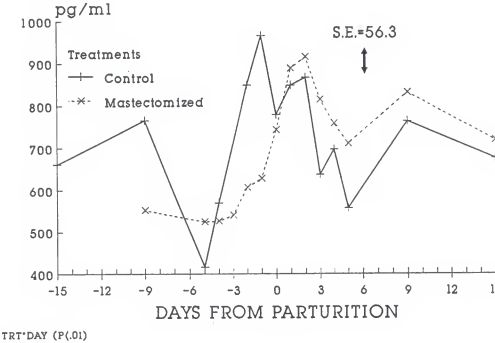
TRT (P(.06)
Figure 10.



TRT (P(.01)
Figure 11.



TRT*WK (P(.08)
Figure 12.



TRT*DAY (P(.01)
Figure 13.

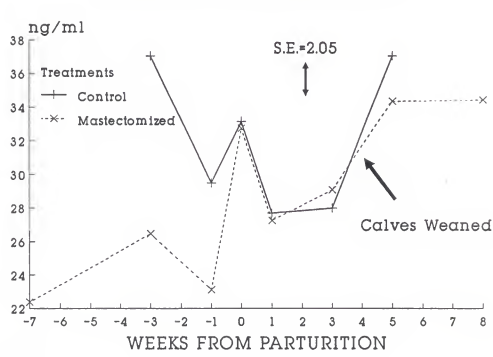
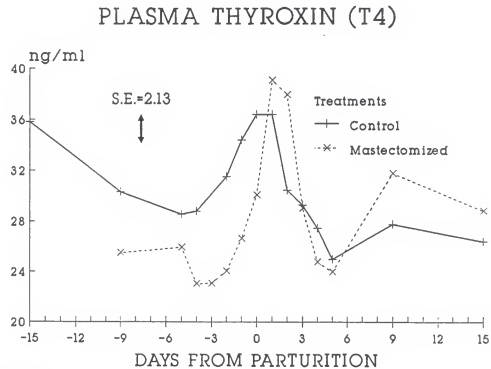


Figure 14.



TRT*DAY (P(.05)
Figure 15.

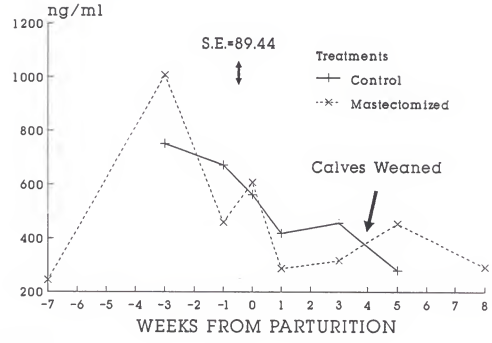


Figure 16.

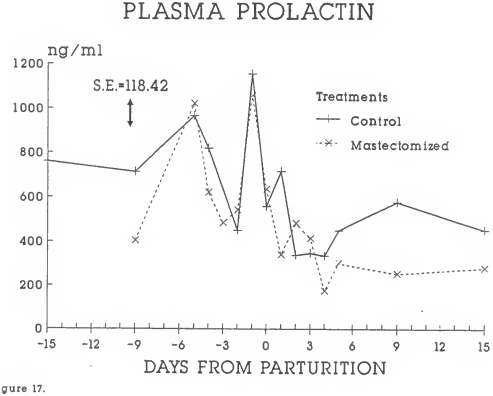
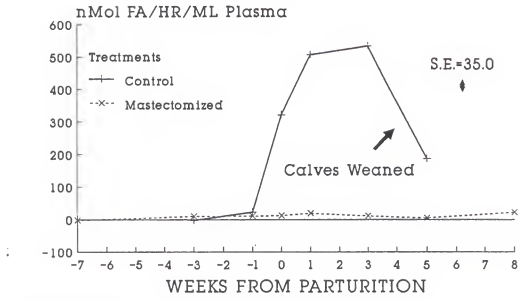


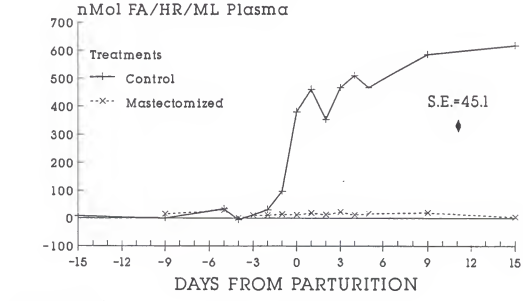
Figure 17.

MAMMARY - JUGULAR
LPL



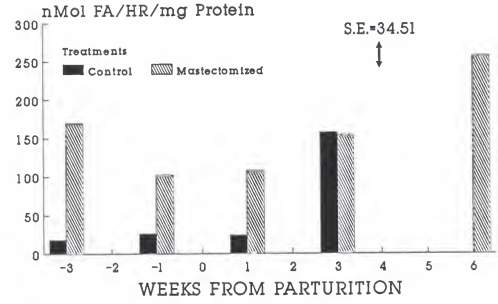
TRT*WK (P(.0001)
Figure 18.

MAMMARY - JUGULAR
LPL



TRT*DAY (P(.01)
Figure 19.

ADIPOSE TISSUE
LIPOPROTEIN LIPASE



TRT (P(.05)
Figure 20.

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Appendix A. Concentration of standard (ug/ml) in heptane).[a]

Fatty acid	ug/ml
C 8	35
C 10	25
C 12	240
C 14	85
C 16	185
C 16:1	15
C 17 (internal standard)	275
C 18	85
C 18:1	265
C 18:2	50
C 18:3	15

[a]Supelco, Inc. Bellfonte, PA. (RM-5, RM-6, and C 17:0).

Appendix B. Composition of the TG premix was:

--0.64 ml glycerol tri(9,10 (n) - ^{3}H)oleate[a](Amersham TRA 191. >99% TG. 5mCi/25 ml hexane) This was diluted to achieve 88x10^{6} DPM. Radioactive decay was calculated based on age of purchased stock and its half-life. This was stored at -20°C under N_2.

--1.00 ml triolein (Sigma T7140. MW=885.4. Approx. 99%) Purchased stock was diluted with toluene to yield 150mM solution. This was stored at 4-6°C under N_2.

Composition of the fatty acid (FA) premix was:

--0.74 ml (9,10(n) -^{3}H) oleic acid[a](Amersham TRK 140. 5mCi/.25 ml hexane) This was diluted to achieve 3.52x10^{6} DPM. Radioactive decay was calculated based on age of purchased stock and its half-life. This was stored at -20°C under N_2.

--1.00 ml triolein

[a]Both the TG and FA radiolabeled standards were purified (Carroll, 1961) with a silica gel column (Baker 7086. J.T. Baker Chemical Company, Phillipsburg, NJ 08865). Columns were conditioned with 3-4 ml toluene. Columns were loaded with 3 ml (1 ml x 3) of respective standard. The eluting solvents were: toluene (1 ml x 3), 20% ether in hexane (1 ml x 5), 2% methanol in ether (1 ml x 5), and ethyl acetate (1 ml x 5). 90.81 and 94.86% of total TG and FA radioactivity was recovered in 20% ether in hexane and 2% methanol in ether eluent, respectively.

Both premixes were evaporated under N_2. Working premixes were made by adding:

--2.5 ml heat denatured serum (HDS). Serum from a lactating cow (#923) was heated at 56°C for 1 h. Serum was centrifuged at 48,000 x g for 20 min. Supranatant was stored at -80°C.

--8.1 ml 8% BSA, .24 M tris, .15 M NaCl, pH 8.6

--0.9 ml 1% triton X-100

Premixes were placed on ice and sonicated (power setting = 4) 4 x 1 min with 1 min intervals for cooling. Premixes were stable on ice for 6-12 h (25).

Appendix C. Calculations used in LPL assay.

1) FA recovery factor =

(DPM FA premix standard x 10)/(DPM FA recovery)

2) nmol FA/DPM =

$$\frac{\text{(FA recovery factor)x(391.3 nmol FA/10 ul premix)}}{\text{(DPM of TG premix standard)}}$$

3) nmol FA/tube =

((DPM/tube) - (DPM/enzyme blank)) x (nmol FA/DPM)

Appendix D. Procedure for preparing adipose tissue for subsequent protein and LPL analysis.

1. Weigh and record approximately 1-3 g of frozen adipose tissue into 50 ml polyethelene centrifuge tube.
2. Keep sample frozen until ready for homogenation.
3. Add approximately 3X ml (1:3 - W:V - Adipose tissue:0.15M KCl) of 0.15M KCl (0-5°C) to tube and homogenize (Polytron) for 30-45 s in ice water.
4. Centrifuge at 900 x g for 10 min at 4°C.
5. Pour fluid through 2 layers of cheesecloth.
6. Freeze filtrate until needed for protein or LPL determination.

STUDIES RELATING TO THE METABOLISM OF THE PERIPARTURIENT AND EARLY LACTATING BOVINE

I. Manipulation of Energy Availability for Milk Synthesis in Early to Mid Lactation Holstein Cows Fed Monensin

II. Metabolic Changes of the Periparturient Bovine

by

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ABSTRACT

Various dosages (0, 150, 300, 450 mg/d) of monensin supplementation were fed to two Holstein cows (trial #1). Changes were recorded in most of the economically important parameter after the initial (150 mg) level was fed. Milk yield increased 6.3%, while fat content and yield decreased 8.6% and 2.8%, respectively. Protein yield decreased 14.9% in early lactation due to monensin and did not change with monensin supplementation in late lactation. As a result of trial #1, four of eight Holsteins at peak lactation (35.6 d postpartum) were supplemented with 200 mg/d of monensin for 3 wk. Net results of monensin supplementation (average of 150 to 450 mg level) include: +7.4% milk yield, +3.6% fat corrected milk, -8.5% fat content, -1.1% fat yield, +0.2% protein content, and +10.1% protein yield. Dry matter intake decreased 1.8%, while feed efficiency increased 5.7%. Milk fatty acid composition and plasma lipoprotein lipase changes reflected stage of lactation shifts and were not effected by monensin in either trial. Ruminal propionate and isobutyrate increased (16.4%, 11.1%, 10.7%, and 8.1% in exp. 1 and 2, respectively) with monensin supplementation.

Mastectomies were performed on eleven beef cows to determine the mammary gland's contribution to homeorhesis during parturition. Fourteen normal beef cows served as controls (C). Plasma glucose tended to increase to a peak earlier in the mastectomized (M) group, and did not decline to prepartal levels as soon as C. Higher glucose levels in M resulted from higher glucagon levels before calving, and caused increased insulin levels after calving. Plasma cortisol was

elevated in M throughout the trial, with inconsistent surges -4 to 5 d from calving in both groups. This suggests that the surges are stress related responses of parturition. Thyroid hormones followed similar patterns between M and C, but the peak associated with parturition was delayed 1 to 3 d in M. This indicates that the mammary gland has a secondary effect on the timing of thyroid hormone release. A plasma prolactin surge at parturition was recorded in both groups and decreased to below prepartal levels in M and C. Mammary lipoprotein lipase activity drastically increased 1 d prepartum and peaked 2 wk postpartum in C, while M remained at undetectable levels after calving. Mammary lipase is independent of direct hormonal control. Adipose tissue lipoprotein lipase declined as a result of parturition in M, indicating peripartal hormonal control. Adipose tissue lipase activity began increasing from minimal levels 3 wk after calving as a result of increased dietary energy intake.